

## Title

Polyketide Synthase Gene from *Sorangium cellulosum*

## Cross-Reference to Related Applications

The present application is a continuation-in-part of allowed U.S. patent application Serial No. 09/144,085, filed 31 Aug. 1998, which is a continuation-in-part of U.S. Patent No. 6,090,601, each of which is incorporated herein by reference.

## Field of the Invention

The present invention relates to the fields of molecular biology, chemistry, and medicine.

## Background of the Invention

*Sorangium* species produce a variety of useful polyketides, including epothilone, myxothiazole, and soraphen. U.S. Patent Nos. 5,962,290; 6,066,721; and PCT patent publication Nos. 98/49315; 99/0398600/24907; 00/31247; and 00/44717 describe methods for making novel polyketides by combining portions of two or more polyketide synthase (PKS) genes to create novel genes that encode a hybrid PKS and by providing synthetic biosynthesis intermediates to modified PKS enzymes. There remains a need for new polyketide synthase genes for use in the preparation of hybrid PKS enzymes and the polyketides produced by such hybrid enzymes. The present invention meets that need by providing recombinant DNA compounds that comprise all or a portion of a PKS gene from *Sorangium cellulosum*.

## Summary of the Invention

The present invention provides recombinant DNA vectors and host cells that comprise the *tmbA* genes of *Sorangium cellulosum* or fragments of those genes.

These and other aspects of the invention are described in more detail in the following description and claims set forth below.

## Brief Description of the Figures

Figure 1 provides a physical map of the *tmbA* gene cluster and an alignment of the cosmids of the invention (34-7, 28-26, and 14H12) that comprise the *tmbA* gene cluster genes and gene fragments. The PKS genes are designated *tmbA*, *tmbB*, and *tmbC*. Open reading frames (ORFs) are designated 1 through 8, inclusive. ORFs 1 and 3 are overlapping. ORF 1 is a thioesterase (TE); ORF 3 is a methyltransferase. ORF 4 is a hydrolase. ORF 6 is an epoxide hydrolase.

Figure 2 provides a structure of the polyketide tombamycin produced by the TmbA PKS in monomeric and dimeric form. R is a substituted or unsubstituted C<sub>3</sub>-C<sub>8</sub> alkyl or cyclic alkyl.

## Detailed Description of the Invention

The present invention provides recombinant DNA vectors that comprise all or a portion of any of the genes in the *tmbA* gene cluster. The *tmbA* gene cluster is comprised of PKS genes *tmbA*, *tmbB*, and *tmbC*, and ORFs 1 - 8, inclusive. Each PKS gene in the cluster is composed of one or more PKS modules, each comprising an acyltransferase (AT), ketosynthase (KS), and acyl carrier

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protein (ACP) domains and optionally one or more ketoreductase (KR), dehydratase (DH), and enoylreductase (ER) domains as well as linkers that connect one domain to another and one module to another. The boundaries of each of these domains can be identified by sequence comparison with known PKS genes and enzymes. In one important embodiment, the invention provides recombinant DNA vectors that encode all or a portion of one or more of these domains that are useful in the construction of hybrid PKS genes and enzymes.

The sequence of the *tmbA* gene cluster is shown below.

CTCCAGATCGACCTGCATGATCTTGCCGACGAGCTGCAGCAGCTCAGGGTCCTCCTGGAT  
CACACGATCGAACGCCCGTACCTCTGCTGTACACGCCGAGGAACTCGCTCGCGGGGAG  
GTGAGAGATCGGCGGCAGGCGGGCGGGTACGTGAGGGGCTCTGCTCGCGGCGACGACCAT  
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CGTCCCAGGCTGTATCCAAAACACGCGAACGGCAGGTCGAGCATGTCATCCAGCCCCCG  
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TCGATATATGGCCCCGCCGGCGCCGGCGTAGGGGAAGCAGAAAAGGCGAAGCTTCGCGTC  
CGTCCGCGCGGTCCAGTGGCAGCAGCCAGGGATTCTTCTCCATGTAGACCTCCGGTGCAAG  
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CTCGTCCGGTATCTCAGGTCCATTGACGAGGCAATGTATTCTATGTTAGGTGTTCCCATAG  
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The sequence of each of the ORFs in this gene cluster and the translated amino acid sequence of the proteins encoded thereby are shown below.

orf1 partial sequence bases 522-1



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GenBank accession number



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SPERPLYLGSSKSNLGHAAAGVAGVIKMLVLAHQHEVLPRTLHAEQPSPHIGWEGSGLS  
LLQEARPWRNRVRRAGVSSFGISGTNAHIILEEAPAEARREPVEAEAPALLPLVLSG  
RDEASVAAQAERWAKWLEEHAEVGWSDVVRTAALHRTHFASRASVQAASVSEAVEVLRAL  
SQGRGHRAVSAGTARARGKVVFVFPQGSQWPGMGRALLEQSAFAEAVQACDEALRPWT  
GWSVLSVLRGEAGEAGEEQPSLERVDVVQPALFAMCVGLAAAWRSLGLEPAAVVGHSQGE  
VSAAVVCGALS LAEGARVVALRSQAVRQQSGMGAMMLVERPVSEVQEHIAPYGEALAAIAA  
VNTSSSTVVS GDVEAVDGLMVELTAEGVFCRKVNVDYASHSAHMDALLPELGAKLSSLRP  
KATQLPFYSTVTGEVSRGEALDGEYWCRLRQTVRLDRALSKLLEDGHGVFVEVSAHPVL  
AMPLTTACGEAQGVVGS LQRDEGGLS QLYRTLGLHVQGHVQHEVDWARVLSGHGGGVVELP  
TYAFQRQRYWLDISKARSDVSSAGLKA AAHPLLGAATKLAEGDGHLFTGRLSLGEHAWLR

GenBank accession number

DHEVFGNVVFPGAGMLELALAAGR TVGSGALSEMVLAEPLVLAEDVAVRLQLSVGAPDAA  
 GRREFGLYSQLEQGPEDAPWVQHATGVLADEPRGI PGELDELATWVPVPGAEEVDLSGFYE  
 RLREGLHYGPAFQGLVELWRRGTTLFGRVVL PKAAGDSAEDYGVHPALMDAALHTMVAA  
 LSERPGANAVLLPFAWSDVLLAMGASELRVHVDLQDHGAEQAMASLYVADSVGQLVVISI  
 GELKLRWATAEQLREATRAEAQHLYRVNFRPVRLVDGSSSESAPPMLVIVSEGGQLAEIL  
 EAEAVASLDVLLARLAQGASAPVRLVLDATAANAGRSPVAASHEAAQEALSLLQAWLSEP  
 RLEGVELAVWTRDAVSAAPGDGVQDLAHAPLWGLVVRTARSEHPERQLRLIDVGTEPVDGG  
 LLERALATATEPELALRGGAAASRLVVRVQAVEEVTRTRGLDPAGTVLVTGGTGELGQAV  
 AEHLVRAHGVRHLVLTSSRGGLEAPGARELVQSLEKLGAEVTVAACDVSKREEVAQVLAG  
 IEAAHPLTAVLHLAGVLDGVLSSQTPERISR VFAPKVDGALHHLHEL TRELDLSAFVLFSS  
 SAAGTLGTSGQSNYAAANSFLDALAAHRRSRGLAATSLAWGSMPEPTGTARPSTAEPGA  
 TKQPGLMPSFSAGLSLLDATLSRPEANLVPAYLDLARLQRGVEASGELPALLRALLRPG  
 LRKAAFQAQEASALRERLARLPESERLNALVALVQAEVATVMGLPRSEAVAADQVLKELG  
 LDSLMAVALRNRLTARTETPLPATLVFDYPTPRAVAELLLKQAFSELKSAGARPRGRRRG  
 QEDEPIAIVSMACRLPGGVATPEDYWRLLAEGKDAIERF PARWDALSIYAPDPDAVGKSY  
 AREGGFVHGVDLFDAGFFGISPREAQAMPQQRLVLETAWEALERAGVRPSALSGSATGV  
 YLGSAGSDYGSQIGSALDALDGYQMTGNLGSVISGRVAVVGLGQPAITVDTACSSSLVS  
 LHLACTALRQGECDLALAGGVVMSTPALFVEFSRLKGMARDGRCKSFSAQADGAGWAEG  
 CGMLLLKRLSDARRDGRVLRGSAVNQDGRSQGLTAPNGPAQQRVIRQALSSCRLSP  
 EDIDAMEAHGTGTS LGDPIEAGALAEVFGPERSPERPLYLGS SKSNLGHGTGPAAGVAGVI  
 KMLVALQREVLPKTLHAEQSPHIAWEGSGLSLLQEARPWRNRGRVRRAGVSSFGISGTN  
 AHIILEEAPAEARRAPVEVKAEVAPAAMPLVLSGRDEAAVNAQAGRWAKWLEGHAEV GWS  
 DVVRTAALHRTHFASRASVQAASVSEAVEVLRALWQGRGHRAVSAGTARARGKVVVFVPG  
 QGSQWPGMGRALLEQSAFAEAVQACDEALRPWTGWSVLSVLRGDGGEQPSLERVDVQ  
 PALFAMCVGLAAAWRSLGLEPAAVVGHSQGEVSAAVVCGALSLAEGARVVALRSQAVRQQ  
 SGMGAMMLVERPVSEVQEHIAPYGEALAAIAAVNTSSSTVVS GDVEAVDGLMVELTAEGVF  
 CRKVNVDYASHSAHMDALLPELGAKLSSLRPKATQLPFYSTVTGEVSRGEALDGEYWC RN  
 LRQTVRRLDRALSKLLEDGHGVFVEVSAHPVLAMPLTTACGEAQGVVVGSLQRDEGGLSQL  
 YRTLGLQLVHGHEVDWARVLSGHGGGVVELPTYAFQRQRYWLDISKARSDVSSAGLKAAA  
 HPLLGAATKLAEGDGHFTGRLSLGEHAWLRDHEVFGNVVFPGAGMLELALAAGR TVGSG  
 ALSEMVLAEPLVLAEDVAVRLQLSVGAPDAAGRREFGLYSQLEQGPEDAPWVQHATGVL T  
 DEPRGIPGELDELATWVPVPGAEEVDLSGFYERLRERGLHYGPAFQGLVELWRRGTTLFGR  
 VVLPKAAGDSAEDYGVHPALMDAALHTMVAA LSERPGANAVLLPFAWSDVLLAMGASEL  
 RVRMELQETADSRQITASLSVADAIQOPAASV GELQLRWATAEQLRAAIRTEAQHLYRVD  
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 VTAAATSQPGVPAASHEATQEALSLLQAWLSEPRLEGVELVWVTRDAVSAAPGDGVQDLA  
 HAPLWGLVVRTARSEHPERQLRLIDVGTEPVDGELLARALATATEPELALRGGAAALARLV  
 RVPAAAETLTPARGLDRGTGLVTGGTGELGQAVAEHLVRAHGVRHLVLTSSRGGLEAPGA  
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 PERISR AFAPKVDGALHHLHEL TRELDLSAFVLFSSMSGTLGTSGQSNYAAANSFLDALAA  
 HRRGCGLAATSLAWGFWAQAGVGMTAHLGEAELSRIRRAGLVPMSEEGLSLLDAALLRA  
 EASLAPVRFDLATLQRKLDTGALPPLFLALLR PGLRRVSPASQGTSAIRERLVALPEQE  
 RLKSLVALVQAEVA AVLGLQGAATIRADQPLLELGMDSLMAVELRNRLSSLI GATLPVTV  
 AFTHPDSRSIGAFLLDMLPSAGERSLPRNDKVAGKWLRI LRSRVPVAVRIVCFPGAGGAA  
 SLFLPLAQHVADDVELVAIQAPARGDRLAETS VTDMSVVFVSEVCDALRGRDLPTLFFGY  
 SFGTWTAYAVLCGALS RGVHRAPLGLAVACMTPPSDARQRTMQLGIEEDDDTVVKRMVAV  
 GAWPEAALDDGELRAALLPSFRADARLGMSYRWAE EKLLDVPVLAVAATRDEL SQMRHPS  
 RRGVR

orf5a

GTGGGCCGAGGAGAAGCTGCTGGACGTCCCTGTCTCGCCGTGGCAGCGACGAGGGACGA  
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GAGCTGTCGACGTGCGTGGAGACGCAGCTGTGGCCAGGGAGAGAGCGCTCGAGCGACCGC  
GCCACCCAGGCACCCGGCGACAGCCGCGGGACGGCGCGGGCCGGTGTGACGGCATG  
CCGCCGCTCGGAGAAGAGCGCGAGAGACCGGGCACCAGCCCATCGCAAGCTTACGTGGGC  
TGA

VGRGEAAGRPCPRRGSDEGRAIPDASSIEAWRQVTTGDFVMSHLDGTHSLVLDDPASLAR  
ELSTCVETQLWPGRERSDRATQAPGDSRGDAAAGVDGMPPLGEERERPGTSPSQAYVG

orf5b

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CCGTCCCCGCGGTGTGCGCGGGTGCCTGGGTGGCGCGGTGCTCGAGCGCTCTCTCCCT  
GGCCACAGCTGCGTCTCCACGCACGTGACAGCTCTCGTGCCAGGCTCGCAGGATCATCC  
AGGACGAGCGAGTGCGTGCCGTGAGGTGGCTCATGACAAAGTCTCCTGTGTCACCTGA

VPGLSRSSPSGGMPSTPAAAPSPRLSPGAWVARSLERSLPGHSCVSTHVDSSRARLAGSS  
RTSECVPSRWLMTKSPVVT

orf 6

ATGCTGCCAAGGAACGACATCATCCATGCTCATGCCGACGTGAACGGCGTCCGCCTCCAT  
TACGCGTCCAGGGGCGCGGGCAAGCTCATCCTGTTATCCACGGCTTCCCCGAGCTCTGG  
TACGCCTGGAAGCGGCAGCTCTTCGATTTCCGGCCGCCACCACCGCGCGGTGGCCCTGGAC  
CAGCGCGGCTACAACCTGTGTCGAAGCCGTCCGGCCGTGGACGCCTACGGCATCGACCTC  
CTGGCCGCGGACATCGCGGCGCTGATCGAGCACCTCGGCGAGGAGAAGGCCGTGCTCGTC  
GGCCACGACGTGGGCGCGGTGCTGGCCTGGGCCGTGGCGCTCCGCCACCCGGAGCGCGTC  
GAGAAGCTCGTCGCCATCAACATGTCTCACCCGGCGTGCTTCGACCGCGCGCTGCGGGAG  
GACCCGTGCGAGCAGGCCGCGAGCCAGTACATGCACCTCTTCCGGAGCCGGTCCGCAGAG  
GAGCACCTCTCCCGGAACGAGTACGGCTTTCTGCGGGAGATCTTCTCGAGCCAGGGCTG  
TCGCAGGGATATTTAGCGAAGCGGACGTCCGTGTCTACCTGGAGGCGTTCGCGCAGCCG  
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GGCCAGCCCGTCCGGGGGAGCAACCTCACCCGGGGTCTCCGCTCGCTCACCGTCTCCGTG  
CCTGTCCTCGTGATCTGGGGCGAGCGGATCCGTACCTCTTGCCCGCGAGCAACCTCGCC  
GGGATCGAGCTTTACGTCCCCGATCTGAGGGTATGCGCGTGCCCGAGGGCAGCCACTGG  
ATCGTCCACGAGGAGCCCGAGATCGTCAACGCGGCGATCCGCGGCTTCTCCTCGCGGCTAG

MLPRNDI IHAHADVNGVRLHYASRGAGKLILFIHGFPELWYAWKRQLFDFGRHHRAVALD  
QRGYNLSSKPSAVDAYGIDLLAADIAALI EHLGEEKAVLVGHVDVGAVVAVALRHPERV  
EKLVA INMSHPACFDRALREDPSQQAASQYMH LFRSRSAEHL SRNEYGFLEIIFLEPGL  
SQGYFSEADV RVYLEAFAQPGAITGGLNIYRAAQI GPPPGQPVGGSNLTRGLRSLTVSV  
PVLVIWGERDPYLLPASNLAGIELYVPDLRVMRVPEATHWIVHEEPEIVNAAIRGFLAR

orf 7

ATGAACACGACGCTCAAGCTCCACGAGGAGTACCCGCCGCCCGGCGAGGAAGACAGCATC  
CGCCAGATCACCGAGATCATGAGGCGCAATTACGAGCAAGCTTACCCCGCGGGCGCCTCG  
CCCGCGCTCCGCGGGGTGCACCCGAAGTCGCACGGCTGCGTGAGGGCCCACTTCGTGCTC  
GACGAGGGCTTGCCCCGCGAGCTCCGCCATGGCGTCTTCCGGGAGCCGCGCGTCTACCCG  
GCGTGGGTCCGGTCTCGTTCGACCTCCTCGCGCGTCCAGTCGGACATGAAGCGCGACTCC

FORBIDDEN TO REPRODUCE

CGCTGCATGGCGATCAAGCTGCTCGGCGTCGAGGGGGAGAAGATCCTCGACGGCGAGAAG  
GACGCGACGACGCAGGATTTCTGATGGGAAACACGGACGTGTTCTTCTCGCGGAACATC  
GCGGACTACGTCGAGCTCATGTCTGCCATGAGCGCGGGCAAGCCCCTCTCGTATTTCTGC  
TCGCTCCGCCCCGCCCTCCGGCTGCGCGAGCTCATGAACTACCTCTCCGTCTGTGCTC  
AAGCCGGTGAAGAACCCCTGCACGCCCGGTATTTACGCCAGACGCCGTTCCGGCTCGGC  
GCGCGGGCGATGAAGTTCTGCGTGGTCCCCCGGCCCTGCGGCGCGCCCGGCGTCCGGGGT  
GAGCCGGGCGACGACGCGCTCAAGCAGGCGGTGGCGCGGCAGCTCGAGGGGGGAGACTGG  
ATGTTGCACTTCTCGTGCAGCTCCAGGCCACCCGACGAAGACGCCCATCGAGGATCCG  
ACGATTCGCTGGAGCGAGGAAGTGTGCGCGTTACCAAGGTCGCGACGATGGTCATCCCG  
GCGCAGCGCTCGACCTCCCGGGACAGGCGGAGTTCGAGGAGAACCTCTCGTTACGCCC  
TGGCAGCCCTGCCGGCGACCGGCCGCTCGGCGGGTGAACCGCGCCCGGCGCGCGGTC  
TACGAGGCGATATCGAAGCTCCGCCACGAGAGGAACGGGGCTCGCCGCGAGGAGCCGGCG  
GCGCCGCGCGCCGGGCGCGCGCGCGCGCGCTCGAGCGCAGGGCCGGCGCGGCTCGGACTC  
GGGTGA

MNTTLKLHEEYPPPGEDSIRQITEIMRRNYEQAYPAGASPALRGVHPKSHGCVRAHFVV  
DEGLPRELRHGVFREPRVYPAWVRFSSSTSRVQSDMKRDSRCMAIKLLGVEGEKILDGEK  
DATTQDFLMGNTDVFFSRNIADYVELMSAMSAGKPLSYFCSLRPPRLRLRELMNYLSVVL  
KPVKNPLHARYFSQTPFRLGARAMKFCVVP RCGAPGVGVEPGDDALKQAVARQLEGGDW  
MDFDLVQLQAHPTKTPIEDPTIRWSEELSPFTKVA TMVI PAQRDLPGQAEFEENLSFTP  
WHALPAHRPLGGLNRARRAVYEAI SKLRHERNGARREEPAAPRAGRARAPSSAGPARLGL  
G

orf 8 - partial sequence

CGGCCGAATTAACCCTCACTAAAGGGATCATGCTCACTGCGAGCCTGTTCTGTGAGCGCG  
GTCGTGCAGGTGATCGTCAACGTCGGACGCTACCGTTCCTGCGTCTCTCGTCCACATGCTG  
CGGTGCTCATGCACGGCTTCCGCGCGCTCCACAAGGACCCCGACGTCGTGATCCGGCG  
ACCGGGCGCCCGGCGCATGGCGCGCTCGCCAGGAACGAAGCCTGTGAAATGCGCTTCGTG  
CACGAGGTGCTCTCCGATCCGGTCTGACGCGCGCAGCGCGAAGCTCGCGTACGGCTCG  
GACCCCGAGCGGCTCGCGGGCCTCGTCGAGTACATCGACGTCGAGGACCTGATCAGCATC  
GCCACCGGCGCCTCGGCGCCGGCCGACGGGGACGGGCGCGAGCTCCTCTTGCCGAGCCGC  
TGGGTGAGCTGCGGCCACGGCGGCTCCAGCGCGCCGAAGCGCTACGCGACGCTGGAGCAG  
TTCTCCACGTACGTGCGCCGCTGGTTTCCCACCCTCGAGGGCACGGCGTCGACAGCCTC  
AAGCTGCGCGTCCGGCGGCTGACGCTCCTCGTCTCGATGATGGTGGTCATCCTCTTCAAC  
TTCGACGGCTTCCAGGTGCTCGCGCGGCTCCACCAGAGCGGCGCCGCGCGCGCAGGTG  
GCCGCCAGGCGGACACGGTGGCGACCACCGCGGCGCGCCTGGGCGAGCTCCCGGCCGGC  
GCGCCGCGGAGCTCCCGACGCCACGCTCGAGGAGCTCGGGCTCGAGATTCAGAAGACG  
GCGACCCTCCTCGACGAGGCGAACCTCGGCGTGGGCTGGCAGCAGAGCTGGATCGTCCAG  
CGGTTCCGCGCGTACCGCCACGATATCCTGGCGCCCCGCGACCTCGTGGAGCTCCTC  
CAGGACACGCTGTTCTGGCTGGCCGGGCTCGCCTTCTCGTGGGGTTGCTCTCCCTGGGA  
GCGCCCTTCTGGGTACCACGTTGCTCGGCTCATCCAGATGCGCAACGAGGTGCAGCAC  
CGGAAGCGCCAGGAGAGCGCGTCCGGCGTGAAGGTGCGGAGCACCGCCCTGCCGTTCCCC  
GCGCGGAGCGCGCCGTCGCCAAGCCTTCTCTGA

RPQLTLTKGIMLTASLFSVAVVQVI VNVGRYRSCVLVHMLRSLMHGFRLHKDPD VVDP  
TGRPAHGALARNEACEMRFVHEVLSDPVLHARS AKLAYGSDPERLAGLVEYIDVEDLISI  
ATGASAPADGDGRELLLP SRWVSCGHGSSAPKRYATLEQFSTYVRRWFPTLEGTASQTF  
KLRVRRLTLLVSMVVI LFNFDGFQVLARLHQSGAARAQVAAQADTVATTAARLGELPAG  
APAE L P D A T L E E L G L E I Q K T A T L L D E A N L G V G W Q Q S W I V Q R F R A Y R H D I L A P P P T S L E L L



QDTLFWLAGLAFSCGLLSLGAPFWVTTFARLIQMRNEVQHRKRQESASGVKVASTALPFP  
ARERAVAKPS

The genes of the *tmbA* gene cluster can be isolated from the cosmids of the invention shown in Figure 1 or from *Sorangium cellulosum* genomic DNA.

The gene products of the *tmbA* cluster can be used to synthesize the polyketide tombamycin, the structure of which is shown in Figure 2.

Tombamycin can be dimerized to produce the dimeric form of tombamycin, the structure of which is also shown in Figure 2.

The invention having now been described by way of written description those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.

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